The soil microbial foodweb revisited with metatranscriptomics - predatory *Myxobacteria* as keystone taxon?

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Abstract

Trophic interactions in the microbial food web of soils are crucial for nutrient and carbon cycling. Traditionally, protozoa are considered the major micropredators of bacteria in soil. However, some prokaryotes, such as *Myxobacteria* and *Bdellovibrio* are also famous for bacterivorous life style. Until recently, it was impossible to assess the abundance of pro- and eukaryotic micropredators in soils simultaneously. Using a metatranscriptomic three-domain profiling of small subunit ribosomal RNA we investigated the abundance of bacterivores in 28 datasets from eleven European mineral and organic soils of different climatic zones. In all soils, *Myxobacteria* comprised a significant proportion from 6 – 14% of prokaryotic 16S rRNA transcripts and more than 60% of all bacterivores in most soils. *Haliangiaceae* and *Polyangiaceae* were most abundant, while the name-giving *Myxococcaceae* were barely present. Other bacterial predators like *Bdellovibrio* were low abundant. Also Protozoan micropredator 18S rRNA transcripts, e.g. from *Cercozoa*, *Amoebozoa and Ciliophora*, were on average less abundant, especially in mineral soils. *Nematodes* were even less abundant. In addition, we applied a longitudinal approach to identify bacterivores during beech litter colonisation. Here, *Myxobacteria* showed prey-dependent, protozoa-like community dynamics during colonisation. Thus, their broad prey range and high abundance suggests a major influence of *Myxobacteria* on structuring the prokaryotic community composition in soil, and might warrant their classification as keystone taxon. Out results suggest the presence of an ecologically important “bacterial loop” in soil food webs, independent of protozoa and nematodes.

Introduction

Predation of predators on prey is a key process in structuring community composition in ecosystems and in maintaining high biodiversity. Predator - prey interactions and dynamics among animals and consequences for ecosystem functioning have been studied extensively since the early days of ecology. While less visible and thus less acknowledged, predation is not foreign to the microbial world. Eukaryotic as well as prokaryotic microorganisms are known to prey on other microorganisms in marine, aquatic and terrestrial habitats as part of the microbial food web (Clarholm, 1985; Azam *et al*., 1983).

Protozoa are traditionally considered the main microbial predators of bacteria, a notion that stems from the fact that, unlike in bacteria, where it is somewhat “exotic”, predation is a common lifestyle among protozoa. Predatory protozoa are known from both aquatic and soil environments and have been considered a key-component of the “microbial loop” responsible for the remineralisation of nutrients (Bonkowski, 2004; Clarholm, 1985). Whereas protozoa in the aquatic system have been well characterised, both in term of their identity and population size, research in soils has been much more hampered, since no adequate molecular tools have been available for a long time, cultivation is often difficult, and direct microscopic observations are impossible (Geisen *et al*., 2015).

Much fewer prokaryotic species are considered predatory, although a predatory lifestyle in prokaryotes probably evolved prior to its development in eukaryotes. Several bacterial predators have been identified, with more and more taxa exhibiting a predatory lifestyle being recognized recently. These include *Myxobacteria*, *Lysobacter*, *Bdellovibrio* and like organisms (BLO), *Vampirococcus* and *Dapterobacter*, among others (Reichenbach, 1999). Especially the *Myxobacteria*, with

58 their ‘wolf pack hunting’ strategy, are known micropredators since more than 70 years
59 ago and famous inhabitants of soil environments that have been isolated from soils
60 world-wide (Keane & Berleman, 2016; Reichenbach, 1999).
61 It has been until recently impossible to assess bacterial and protist community
62 composition with the same methodology. Although PCR amplicon methodologies
63 enabled the study of both groups separately, a direct comparison of their relative
64 abundances was not possible due to the absence of universal primers that would tackle
65 all groups without bias. However, these obstacles are avoided when applying random
66 hexamer-primed reverse transcription as in metatranscriptomics approaches that target
67 SSU rRNA of organisms from all three domains of life (Urich *et al.*, 2008).
68 Furthermore, these rRNA transcripts are indicative of ribosomes and thus are likely
69 derived from metabolically active cells and can be considered markers for living
70 biomass. The generated cDNA fragments originate from different regions of the SSU
71 rRNA molecule unlike PCR primed specific sites, and are therefore insensitive to the
72 presence of introns or primer mismatches, when PCR primers are applied.
73 We have recently used this PCR-free metatranscriptomics approach to reveal the
74 diversity of the active soil protist communities within five different natural soil
75 systems in Europe, including forest, grassland and peat soils as well as beech litter
76 (Geisen *et al.*, 2015).
77 Here we have focused on other groups of microbial predators - predatory bacteria. We
78 have assessed the relative abundance of SSU rRNAs from bacterial groups known to
79 exhibit a predatory lifestyle in these soils. Metatranscriptomics enabled the direct
80 comparison of SSU rRNA transcripts from bacterial and protozoan micropredators
81 and revealed that potentially predatory bacteria, especially *Myxobacteria*, were
82 abundantly detected in all soils, while protozoa abundances were much more variable.

The underlying causes and consequences for our perception of microbial predation in soils are discussed and an alternative model of the soil microbial loop is put forward.

Material and Methods

Data acquisition

The investigated metatranscriptomes had been obtained from different previous studies on a range of European soils (Table 1). These included 4 samples from organic peatland, 3 samples from organic floodplain, 3 samples from gleic fluvisol, 3 samples from mineral grassland, 2 samples from organic forest litter, 4 samples from mineral forest soil, and 3 samples each from 3 different mineral shrubland soils (Beulig *et al*., 2016; Epelde *et al*., 2015; Geisen *et al.*, 2015; Tveit *et al.*, 2013; Urich *et al.*, 2008) Furthermore, metatrascriptomic data were obtained from four different beech litter types (K, A, O and S), which had been incubated with the same microbial community in mesocosms (see Wanek *et al*., 2010 for details of the experimental setup). Litter samples were taken at three time points: after two weeks and after three and six months after inoculation, flash-frozen in liquid nitrogen and stored at -80 °C. RNA was extracted and double-stranded cDNA was prepared as previously described (Urich *et al*., 2008). 454 pyrosequencing was performed at the Norwegian Sequencing center, CEFS, University of Oslo (Norway). Raw sequence data were submitted to the NCBI Sequence Read Archive (SRA) under the accession number SRP134247.

Bioinformatic analysis

Raw sequence datasets were filtered to a minimum length of 200 – 300 nucleotides and a minimum mean quality score of 25 using prinseq-lite (Schmieder & Edwards, 2011). SSU rRNA sequences were identified via SortMeRNA (Kopylova *et al*., 2012). USEARCH (Edgar, 2010) was used to randomly subsample datasets to a maximum of

50 000 - 100 000 sequences. The datasets were mapped against the silva123.1 database by blastn (Altschul *et al.*, 1990) using CREST (Lanzén *et al*., 2012). The obtained blastn files were taxonomically assigned using MEGAN (Huson *et al*., 2011, min score 155; top percent 2.0; min support 1). The number of SSU rRNA reads of the investigated organisms was normalized in MEGAN to the total number of read counts. Investigated taxa with predatory lifestyle were *Myxococcales*, *Bdellovibrionales*, *Lysobacter*, *Dapterobacter*, *Vampirococcus*, *Amoebozoa*, *Cercozoa*, *Ciliophora*, *Foraminifera*, *Euglenozoa*, *Heterolobosea*, and *Nematoda*. Different Nematoda taxa were not investigated separately. The read counts of each analysed bacterivorous group were then normalized to the prey bacterial SSU rRNA reads.

Results for organic (excluding MO samples) and mineral soils were tested for differentially expressed sequences with the R package edgeR (McCarthy *et al*., 2012; functions glmFit and glmLRT), using the non-normalized total read counts MEGAN file.

Results

Abundance of bacterivores in soil microbiomes

We screened the SSU (16S and 18S) rRNA fraction of 28 soil metatranscriptome datasets obtained from eleven different soils across Europe for bacterivorous pro- and eukaryotes (Table 1). Bacterivore abundance was displayed as the fraction of all bacterial SSU rRNAs (Figure 1a). It revealed that *Myxococcales* SSU rRNA reads comprised a high proportion of all bacterial SSU rRNAs, with 9% on average, ranging from 3.5 to 18.9%, and higher than all other investigated bacterivores. Their highest proportion in relation to bacteria was detected in peat soils. The two other sites that showed abundances above 10% were an organic fluvisol and a beech litter layer. The latter came up as the only exception in the pattern, i.e. here the Protozoa were the most abundant bacterivorous group. Nevertheless, the *Myxococcales* SSU rRNA still comprised a proportion of more than 10% of the overall bacterial reads in these samples. Overall, SSU rRNAs of protozoa were the second most abundant. Like the *Myxococcales* they were generally more abundant in organic soils than in mineral soils. The only two cases where their proportion was above 10% of all bacterial SSU rRNA reads were a peatland and forest litter sample, respectively. While *Myxococcales* abundance never dropped below 3.4%, protozoa abundance was much lower in mineral soils (down to 0.7%). The third most abundant group was *Nematoda*. They showed greater variation in abundance compared to the aforementioned taxa, especially in organic soils, where they showed both their highest abundance, namely in the forest litter horizon, and also their lowest abundance, which occurred in the suboxic mofette soil. This was the only sampling site, where their abundance dropped below 0.1% of the overall bacterial SSU rRNA reads. The only other soils which showed abundances above 1% were the organic peatland samples

and the mineral Rothamsted soil. All mineral soils showed fractions of *Nematoda* SSU rRNAs within 0.1 – 1%. The *Bdellovibrionales* comprised even lower SSU rRNA abundances. Similar to the aforementioned, highest relative abundance of *Bdellovibrionales* was observed in organic soils. We did not detect *Vampirococcus* and *Dapterobacter*. *Lysobacter* comprised the lowest SSU rRNA abundances of all detected micropredators, namely 0.07% or lower.

We separated the SSU rRNA data of potential prey bacteria into gram-positive and gram-negative phyla, respectively. Overall, SSU rRNA from gram-negative bacteria comprised approximately 80%, while SSU rRNA from gram-positive were approximately 20%. The latter were slightly more abundant in mineral soils (supplementary figure S1).

Myxococcales dominate bacterivorous taxa

Comparing all investigated bacterivorous groups, the *Myxococcales* were highest abundant in every sampling site, except forest litter (Figure 1b). In fact, in nine of the eleven sites, including all mineral sampling sites, the proportion of *Myxococcales* SSU rRNAs was more than 60% of all micropredators. Additionally, the only exception where the *Myxococcales* did not account for the highest abundant micropredator was the forest litter sample. Here, their proportion of the bacterivorous groups was below 30%. Correspondingly, the protozoa were the most abundant group in that site, comprising up to more than half of all bacterial predators. However, in all the other sampled sites, the proportion of the protozoa was below 40%, in three cases even below 20%. Those were namely the organic mofette samples as well as the mineral Rothamsted site, and mine M from Spain, where the lowest percentage of all micropredators was observed. All of the sampling sites had *Nematoda* SSU rRNA

below 20%. Their highest proportions occurred in the organic forest litter samples. Moreover, the only other two sites where their proportions were above 10%, were Rothamsted, where they were even more than the protozoa, and mineral mine H. All other sites showed proportions below 10%, with the lowest proportions in samples from mofette. The *Bdellovibrionales* were below 10% of micropredators in all sampling sites.

Community composition of Myxobacteria

We analysed the community composition of *Myxococcales* in more detail (Figure 2a). The most dominant family was *Haliangiaceae, followed by Polyangiaceae* and Blrii41, a family level group in the SILVA taxonomy that is currently devoid of cultured representatives. These three together comprised more than 2/3 of *Myxobacteria* SSU rRNAs in all but one site. *Haliangiaceae* and *Polyangiaceae* were more abundant in mineral soils, while Blrii41 was more characteristic for organic soils. The name-giving family *Myxococcaceae*, which is comprised, among others, of the most frequently isolated genera *Myxococcus* and *Corallococcus*, was barely detectable.

Protozoa community composition

As previously found (Geisen *et al*., 2015), were the *Amoebozoa*, *Cercozoa* and *Ciliophora* three most abundant protist groups the (Figure 2b). While *Amoebozoa* and *Cercozoa* dominated in mineral soils, the *Ciliophora* were most abundant in organic soils. The remaining predatory groups *Foraminifera*, *Euglenozoa*, and *Heterolobosea* accounted for low abundances on average. The mofette R soil samples were an exception, with *Foraminifera* comprising more than 20% of protists.

198

199 *Dominance of Myxobacteria among bacterivores in mineral soils*

200 We compared the average micropredator abundance (normalized to the prey bacteria)
201 between mineral and organic soils (excluding MO samples) based on SSU rRNA
202 reads (Figure 3). *Lysobacter* data are not shown due to low abundances. Remarkably,
203 micropredator SSU rRNAs comprised 26.4% of prey SSU rRNAs in organic soils, as
204 compared to only 7.9% in mineral soils. Although concomitantly lower in abundance
205 in mineral soils, *Myxococcales* comprised the highest micropredator proportions in
206 both soil types (13.5% in organic vs. 5.7% in mineral soil). While protozoa were
207 almost equally abundant in organic soil, they comprised approx. 1/4 of *Myxobacteria*
208 in mineral soils. In fact, the percentage of *Myxococcales* within the bacterivores was
209 significantly higher in mineral soils, i.e. 72% compared to 51% in organic soils. Thus,
210 the decrease in abundance of *Myxococcales* in mineral soils was not as strong as seen
211 in the other micropredators.

212 To statistically verify the observed differences in abundance between organic and
213 mineral soils, we tested the data for differentially expressed SSU rRNAs of
214 micropredators. While the protozoa ($p < 0.01$), *Lysobacter* ($p < 0.01$), and
215 *Bdellovibrionales* ($p = 0.02$) were significantly differently abundant between organic
216 and mineral soils, no significant differences were detected for *Myxococcales*
217 ($p = 0.31$) and *Nematoda* ($p = 0.78$). This supports the observed phenomenon, where
218 the *Myxococcales* remained dominant in mineral soils, while the SSU rRNA
219 abundances of other investigated micropredators significantly decreased in mineral
220 soils.

221

222 *Temporal dynamics of bacterivores during community succession*

223 It has been shown that *Myxobacteria* can have a saprotrophic life style next to
224 bacterivory (reviewed in Reichenbach, 1999). We therefore analysed micropredator
225 dynamics in a litter colonisation experiment. In a longitudinal experiment four types
226 of sterilized beech litter differing in their C:N:P ratio were colonized by the same
227 microbial community taken from beech forest soil (Table 2, Wanek *et al.*, 2010).
228 Metatranscriptome data were obtained from three time points over the course of six
229 months. SSU rRNA abundances of protozoa, *Nematoda,* and *Myxococcales*, as well as
230 total bacteria and fungi were assessed to investigate their temporal dynamics (Figure
231 4). Microbiomes on litters K and S were strongly dominated by fungal SSU rRNAs as
232 compared to bacterial rRNAs, while litters A and O had higher proportions of
233 bacterial reads. The fungal:bacterial ratio stayed rather constant for each litter type
234 over time. SSU rRNAs of bacterivores generally increased in relative abundance over
235 time, especially from two weeks to three months. Remarkably, the bacterivores
236 (including *Myxococcales*) appeared earlier and in higher relative abundance in litters
237 with more prey bacteria. With few exceptions, protozoa comprised the most abundant
238 predator of bacteria, with *Myxococcales* and *Nematoda* being second and third most
239 abundant respectively. It appeared that after three months a rather stable predator:prey
240 ratio had established.
241
242 **Discussion**
243 *Metatranscriptomics-enabled holistic assessment of soil micropredators*
244 It has been until recently impossible to assess soil bacterial and protist community
245 composition with the same methodology. Although PCR amplicon methodologies
246 enabled the study of both groups separately, a direct comparison of their relative
247 abundances was not possible due to the absence of universal primers that would tackle

all groups without bias. The rRNA fraction of metatranscriptomics data enables broad three-domain community profiling of abundant bacteria, archaea, and eukaryotes via rRNA (Urich *et al*., 2008). This innovative approach has constantly been developed further and recently come to maturation due to lower-cost NGS sequencing technologies and bioinformatic tools (e.g. (Bengtsson *et al.*, 2018; Schwab *et al*., 2014; Tveit *et al.*, 2015)). Using this approach, we have recently created a first molecular census of active protists in soils (Geisen *et al*., 2015). The study showed that protozoa usually not detected with general PCR primers such as *Amoebozoa* and *Foraminifera* are abundantly present and provided the most comprehensive picture of active protist communities in soils to date. The strength of rRNA transcripts for comparatively unbiased views into community composition and assessment of unseen microbial diversity has recently gained popularity (e.g. Karst *et al*., 2018).

Predatory Myxobacteria as key-stone taxon in mineral soils?

In all investigated soils the *Myxobacteria* comprised a significant proportion of the overall bacterial SSU rRNA transcripts, with an average of 9%. This confirms a recent PCR / 16S rRNA gene based survey where *Myxobacteria* also comprised a substantial fraction (4.1%; Zhou *et al.*, 2014). In our study, lower micropredator abundances were detected in mineral soils as compared to organic soils, which may be due to less available carbon resulting in lower prey cell density in mineral soils. However, given the predatory traits of most myxobacterial taxa, their high relative abundance hints towards an important role in the microbial food web of soils. Moreover, with only one exception, the *Myxobacteria* exhibited the highest abundances when compared to all other bacterivores.

Traditionally, protists are considered to be the dominant group preying on bacteria (e.g. Geisen *et al.*, 2016; Trap *et al*., 2016). In contrast to this, our data suggest an importance, possibly even dominance of *Myxococcales*. In fact, *Myxococcales* comprised approx. 3/4 of all micropredators in mineral soils. Possibly, the smaller pore sizes in mineral soils provide restricted access of protists to their bacterial prey, as compared to the smaller *Myxobacteria*. Thus, microorganisms inhabiting non-continuous capillary pores could be protected from predation by Protozoa and *Nematoda*, but not from the similarly-sized *Myxobacteria*. The prokaryotes inhabiting the organic soil horizons, with unprotected macro-pore space, would in turn be subjected to higher grazing pressure. The *Myxobacteria* and protozoa exhibit fundamentally different predation strategies, with the much smaller *Myxobacteria* being famous for their social 'wolf-pack' hunting combined with the secretion of lytic enzymes, as compared to the larger phagotrophic protozoa (Reichenbach, 1999). The more similar cell size of *Myxobacteria* and prey bacteria could thus favour myxobacterial predation in mineral soils with small pores. Given the broad prey range of *Myxobacteria*, their high abundance in soils suggests a major influence on structuring the prokaryotic community composition, and might warrant their classification as key-stone taxon.

Interestingly, the majority of *Myxobacteria* was not related to the well-studied and easy-to-isolate *Myxococcaceae*, but belonged to families with a less well characterised prey spectrum, such as *Haliangiaceae*, *Kofleriaceae* and *Polyangiaceae* (see Figure 4), similar to findings of Zhou *et al*. (2014). The data in this study hint toward biases in culturability within the *Myxococcale*s. In fact, one large family-level group abundant in organic soils, represented in the SILVA database by clone Blrii41, is currently without any cultured representatives.

297

298 *A bacterial loop within the microbial loop*

299 The soil survey gave no direct proof of whether the *Myxobacteria* (or any presumed
300 micropredator) actually showed bacterivorous behavior *in situ*. In a colonisation
301 experiment with sterilised beech litter we compared their succession with other
302 bacterivores, such as the protozoa. In general, the abundance of potential bacterivores
303 was positively associated with abundance of prey bacteria, and increased over time
304 indicating a developing food web during litter colonisation. *Myxobacteria* and
305 protozoa abundances developed similarly over time. This observation hints at
306 *Myxobacteria* indeed having a predominantly predatory and not saprotrophic lifestyle,
307 thus rendering the *Myxococcales* a prominent predatory taxon feeding on other
308 bacteria.

309 There is direct *in situ* evidence for myxobacterial bacterivory from RNA-stable
310 isotope probing studies. The hallmark study of Lueders and colleagues
311 (2006) introduced the use of isotopically labelled prey bacteria to target the general
312 diversity of micropredators in soil *in situ* and follow the carbon flow through the
313 bacterial channel of the soil food web. The authors reported the detection of labelled
314 sequences related to *Myxococcus*, *Lysobacter* and *Bacteroidetes*. However, due to the
315 available technology at that time, they were not able to assess the contribution of
316 predatory protozoa. Another shortcoming was the use of *E. coli* cells as prey because
317 the survival of *E. coli* cells added to soil is rather low. In a more recent follow-up
318 study with labelled native soil bacteria, Zhang and Lueders (2017) provided evidence
319 for niche partitioning between bacterial and eukaryotic micropredators in soil, driven
320 by soil the compartment. Interestingly, the *Myxobacteria* preyed on both gram-
321 positive and gram-negative bacteria. Like in their previous study, the relative

322 contribution of pro- and eukaryotic micropredators could not be assessed due to
323 methodological limitations. Another recent study also included fungi and bacteria
324 (Kramer *et al*., 2016), and indicated that the commonly accepted split of energy
325 channels does not exist.
326 The dominance of *Myxobacteria*, especially in mineral soils, suggests their important
327 role in the soil microbial foodweb (Figure 5). The so-called microbial loop in soil
328 (Bonkowski, 2004) is important for the remineralisation of nutrients, where especially
329 protozoa and *Nematoda* feed on bacteria and by this set free nutrients, which are in
330 turn provided for the bacteria as well as plants (Coleman, 1994). Our observations
331 hint at the presence of an ecologically important 'bacterial loop', especially in mineral
332 soils, within the prokaryotes and independent of protozoa, that has been overlooked
333 until today. As a consequence, bacterial micropredators might not only be important
334 for shaping microbial communities but might also prove to be important for the
335 recycling of nutrients in soils, as it has been shown for protozoa (Bonkowski, 2004;
336 Koller *et al.*, 2013), and thus potentially for nutrient and carbon cycling.
337 Although the *Myxococcales* order comprised a significantly high proportion of
338 bacterial SSU rRNA in all sites, differences at family-level were observed among the
339 soils. Nevertheless, the two most abundant groups were the *Polyangiaceae* and
340 *Haliangiaceae*. The high dominance of the latter may well be due to the
341 *Haliangiaceae* taxon being clustered together with the *Kofleriaceae* taxon in the
342 current SILVA database. Interestingly the name-giving group of the *Myxococcaceae*
343 comprised a very low proportion of all *Myxococcales*. The *Myxococcaceae* are known
344 to be easily cultivable from a variety of environmental samples. Our data show that
345 other, less well characterised families are in fact much more abundant in soil. Thus,

346 efforts should be undertaken to investigate their biology and in particular their prey
347 spectrum.
348
349 *Methodological considerations*
350 The micropredator abundance data in this study are derived from the abundance of
351 SSU rRNA in metatranscriptomes. This does not reflect organismic abundance but is
352 rather a proxy of living biomass (Urich & Schleper, 2011). In fact, several factor need
353 to be taken into account when comparing the SSU rRNA from different pro- and
354 eukaryotic organisms. Results of various studies suggest differences in RNA contents
355 per biomass (1) between organisms and (2) between growth phases, respectively. The
356 ribosome density in prokaryotic cells is generally considered higher than in
357 eukaryotes. However, few data are available to our knowledge. The RNA content of
358 *E. coli* was determined to be 15.7% of dry mass (dm) (Stouthamer & van
359 Leeuwenhoek, 39,545-565, 1973), of *Bacillus subtilis* between 8.5% and 14% dm^{-1}
360 (Tempest *et al*., 1968), *Saccaromyces cerevisiae* 23% dm^{-1} (Parada & Acevedo, 1983),
361 *Aspergillus* 5.9% (Carlsen *et al.*, 2000) and *Penicillium chrysogenum* between 5% and
362 8% (Henriksen *et al*., 1996). Furthermore, prokaryotic cells in an exponential growth
363 phase are known to contain more RNA than cells in stationary phase (e.g. Tempest *et*
364 *al.*, 1968). Preliminary data (Petters & Urich, unpublished) hint to correction factors
365 to be applied when comparing rRNA based abundances from metatranscriptomes
366 between pro- and eukaroytes. Nevertheless, a recent study showed that rRNA
367 correlates better with cell counts than ribosomal RNA genes (Giner *et al*., 2016).
368 Thus, our metatranscriptomics data identify the predatory *Myxobacteria* as important
369 players in the midst of the soil food web and suggests a prominent role in the soil
370 microbial loop in particular.

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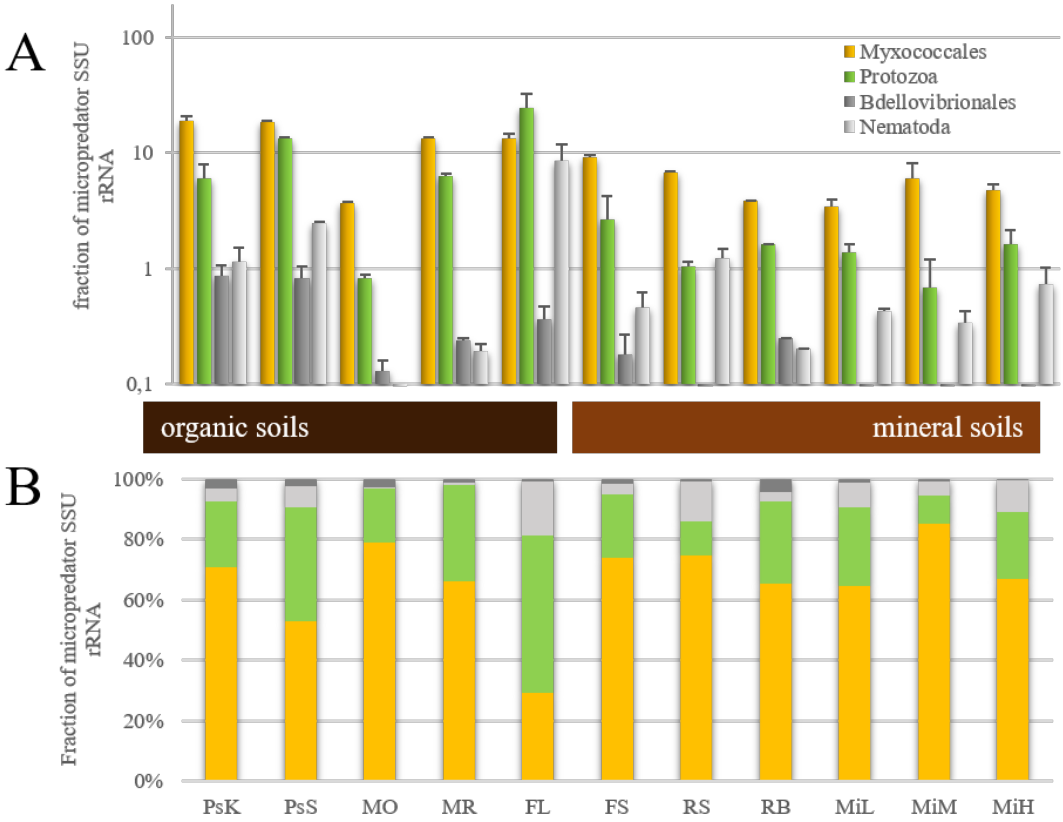
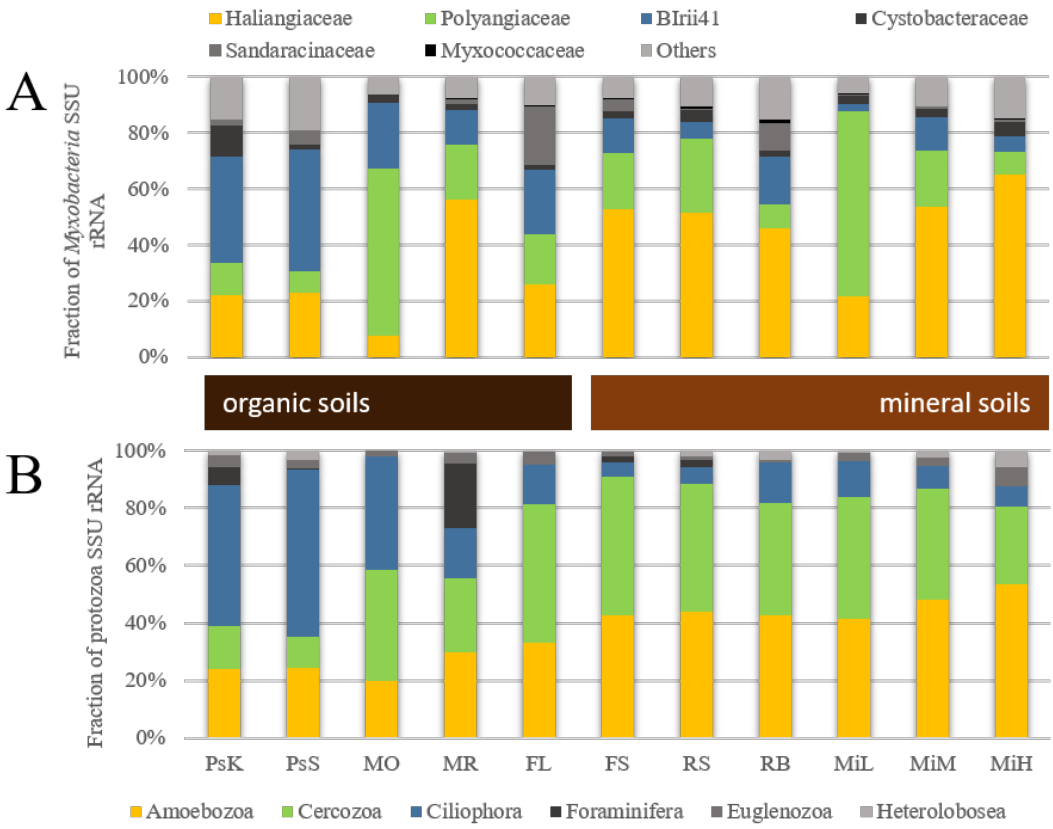
Table 1. Conext data for relevant sampling sites

Site	Peatland soil ”Knudsenheia”	Peatland soil ”Solvatn”	Mofette	Mofette reference	Rothamsted grassland	Rotböhl	Forest Litter	Forest Soil	Mine L	Mine M	Mine H
Abbreviation	PsK	PsS	MO	MR	RS	RB	FL	FS	MiL	MiM	MiH
Location	Ny-Ålesund, Norway (Svalbard)	Ny-Ålesund, Norway (Svalbard)	Hartoušov, Czech Republic	Hartoušov, Czech Republic	Rothamsted, United Kingdom	Darmstadt, Germany	Vienna woods, Austria	Vienna woods, Austria	Coto Txomin, Spain	Coto Txomin, Spain	Coto Txomin, Spain
Climatic zone	Arctic	Arctic	Temperate	Temperate	Temperate	Temperate	Temperate	Temperate	Temperate	Temperate	Temperate
Biome	Fen wet land	Fen wet land	Floodplain	Floodplain	Grassland	Grassland	Temperate deciduous forest	Temperate deciduous forest	Shrubland	Shrubland	Shrubland
Dominant vegetation	Mosses	Mosses	*Filipendula ulmaria*	*Deschampsia cespitosa, Eriophorum vaginatum*	N.A.	N.A.	*Fagus sylvatica*	*Fagus sylvatica*	*Ulex europaeus*	*Festuca rubra*	*Festuca rubra*
Substrate type / Horizon	Organic peat (Top layer)	Organic peat (Top layer)	Organic soil	Gleic fluvisol	Mineral soil	Mineral soil	Litter horizon	Mineral soil (A horizon)	Mineral soil	Mineral soil	Mineral soil
pH	7.3	7.6	4.7	5.3	4.9	7.1	N.A.	4.5-5.1	3.9	5.6	5.9
Moisture (% soil dry weight)	1010	900	N.A	N.A.	33	32	18	43-64	52	49	30
# of replicates	2	2	3	3	2	1	2	4	3	3	3
Sampling time	August 2009	August 2009	July 2013	July 2013	July 2009	January 2006	May 2008	May 2008	March 2011	March 2011	March 2011
Sequencing method	454 GS FLX Titanium	454 GS FLX Titanium	Illumina HiSeq 2500	Illumina HiSeq 2500	454 GS FLX Titanium	454 GS 20	454 GS FLX	454 GS FLX	Illumina HiSeq 2000	Illumina HiSeq 2000	Illumina HiSeq 2000



521 **Figure 1. Screening of pro- and eukaryotic micropredators.** (A) Fraction of major
522 identified micropredator SSU rRNA normalized to SSU rRNA of prey bacteria. (B)
523 Fraction of major identified micropredator SSU rRNA normalized to total
524 micropredator SSU rRNA. Error bars show standard deviation of replicates. For sites
525 see Table 1.



526 **Figure 2. Screening of *Myxococcales* and protozoa taxa. (**A) Fraction of identified
527 *Myxococcales* SSU rRNA normalized to overall *Myxococcales* SSU rRNA. (B)
528 Fraction of protozoa SSU rRNA normalized to total protozoa SSU rRNA. For sites
529 see Table 1.

530

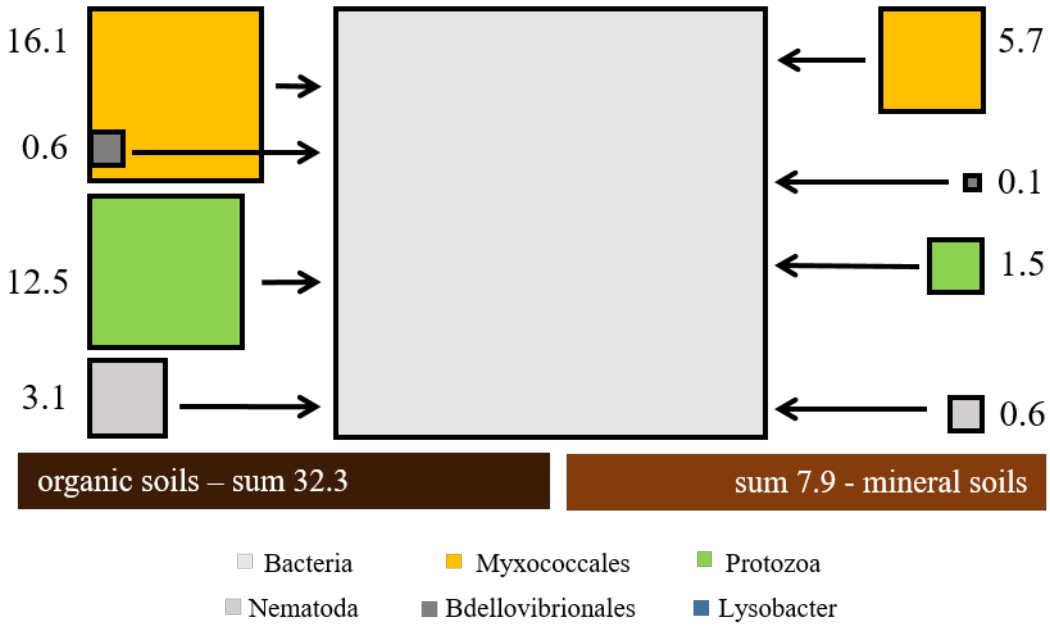
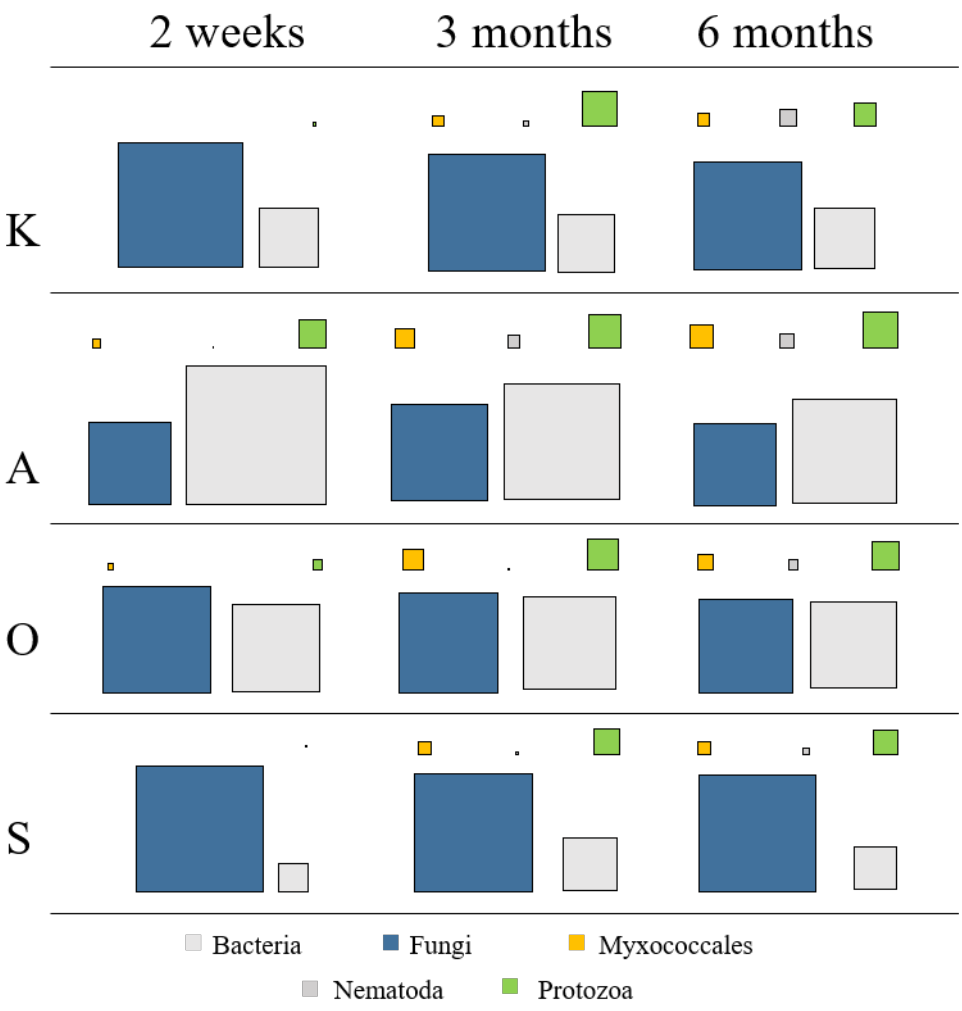


Figure 3. Comparison of organic and mineral soils. Fraction of major identified micropredator SSU rRNA normalized to SSU rRNA of prey bacteria. Average in organic soils (excluding MO samples) on the left; average in mineral soils in the right. Area of boxes resembles abundance of SSU rRNA. Numbers show proportions [%] of prey bacterial SSU rRNA. *Lysobacter* data are not shown due to low abundances.



536 **Figure 4. Colonisation of beech litter.** Fraction of major identified micropredator,
537 fungi, and prey bacteria SSU rRNA. Area of boxes resembles abundance of SSU
538 rRNA. *Lysobacter* data are not shown due to low abundances. For litter types see
539 Wanek *et al.*, 2010.

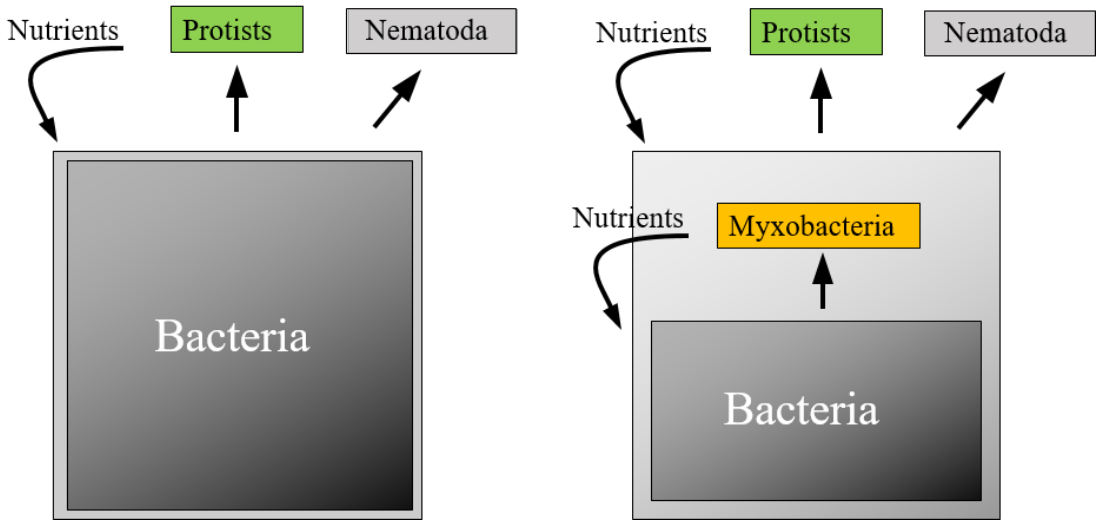


Figure 5. Simplified soil microbial loop. Left: Traditional microbial loop with separate roles of prokaryotic and eukaryotic organisms. Right: Microbial loop containing a bacterial loop independent of eukaryotic organisms. Straight arrows: links between trophic levels. Bent arrows: provision of nutrients.